RESEARCH PROPOSAL

Bioactive products from endophytes associated with

***Albizia lebbeck* (L.) Benth**

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Submitted by

Parikshana Mathur

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Under the Supervision of

Dr. Payal Mehtani
Associate Professor
Department of Biotechnology

Dr. Charu Sharma
Senior Assistant Professor
Department of Biotechnology

DEPARTMENT OF ENVIRONMENTAL AND LIFE SCIENCES

(Department of Biotechnology)

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1. Introduction

Microorganisms are renowned as a prolific source of natural products, many of which are medicinally important products (Huang and Tin, 2017). Recently microbes associated with plants have proved to synthesis products of higher value and medicinal potential (Subbulakshmi *et al.*, 2012). Endophyte is a family of microorganisms that grows in the intra and intercellular regions in the plant tissues without causing any damage to the host plant. Endophytic fungi are reported to produce variety of bioactive metabolites. These metabolites are extracted and used as drugs for treatment against variety of diseases and some of these also find applications in agriculture and food industries (Shukla *et al.,* 2014).

Taylor *et al.,* 1999, have discovered enodophytic fungi from fossils of stems and leaves. Such a long relationship between the host plant and the microorganism has lead to mutual exchange of information and development of a common genetic stem and which helped the fungi in adapting with the environmental stresses more competently. As a result the compatibility of the endophyte with with the host plant increased. In this association the fungi produced certain secondary metabolites which helped in the defense mechanism of the plant and showed protective function against pathogen and insect (Gunatilaka, 2005). These secondary metabolites are classified into various groups like resins, tannins, saponins, terpenoids, phenolic acids, alkaloids, benzopyranones, flavonoids, quinones, steroids and many others (Godstime *et al.*, 2014).

Both plants and microorganisms are benefited from synthesis of secondary metabolites as they help in many physiologic functions which are common to host as well as endophyte. Hence, the utilization of botanical diversity to discover new drugs has led to the discovery of new microbial strains which are able to synthesize compounds which were until now believed to be normal plant products (Nicoletti *et al.,* 2015). Almost all plants are inhabited by endophytic microorganisms which are able to synthesize secondary metabolites. Therefore, scientists consider that these products are a major factor which influences the evolution and establishment of mutualistic interrelations (Schulz *et al.,* 2002).

Nature has blessed human beings with the wealth of plants that are used as cure for various ailments. *Albizia lebbeck,* a native medicinal plant of Rajasthan is also known to harbor endophytes that in some way contribute to the antidiabetic, antiulcer, anti inflammatory, analgesic, antidepressant, etc. properties of the plant. Characterization of bioactive compounds by the fungal endophytes of *Albizia lebbeck* and isolation of such fungal endophytic strains having the potential to produce economically important bioactive compounds is the main aim of this research.

1.1. Alkaloids

Alkaloids comprise the largest class of secondary plant substances and one of the most important fungal metabolites from pharmaceutical and industrial point of view. They are a class of nitrogenous organic compounds and are very important molecules, not only for chemical reasons, but also for their diverse biological properties, such as antifungal, anticancer, and antiviral activities and an important source for drugs (Wang *et al.,* 2011).

1.2. Triterpenoids

The most purified anti-microbial secondary metabolites from endophytes are terpenoids and polyketides (Mousa *et al.,* 2013).

1.2.1. Steroids/Streols

Plant sterols and steroids are compounds that wield a broad range of biological activities like reproduction, plant growth and in dealing with biotic and abiotic stresses. The primary use of natural steroids in health care is to reduce inflammation and other disease symptoms (Rasheed *et al.,* 2013).

1.2.2. Saponins

Saponins are compounds secreted by plants and endophytic microorganisms. Saponins are the glycosides having a sugar moiety bonded with glycosidic linkage to a non sugar portion. The structural complexity in the saponins reflected in their diversity of physicochemical, pharmacological and biological properties and led to the saponins as commercially important and potential compounds with wide variety of applications in food, cosmetics and pharmaceutical/health sectors. Saponins are reported to exhibit activities like antimicrobial, cell membrane perturbing, hemolytic, neutraceutical hypolipidemic, immune modulating, surface active and cell cytotoxicity (Netala *et al.,* 2015).

1.3. Phenolic compounds

The term phenolic compounds includes a wide range of plant substances which are water soluble and have a common aromatic ring having hydroxyl substituents.

1.3.1. Flavonoids

Flavonoids are the largest group of phenolic compounds and are mainly water soluble compounds, structurally derived from parent susbstance flavones. They are found in mixtures of different classes in plant.

1.3.2. Tannins

Tannins are water soluble phenolic compounds of plant origin which are capable of transforming raw animal skins into leather because of their ability to cross link with protein. They are reported to be responsible for decreases in growth rate, feed intake and efficiency, protein digestibility and net metabolizable energy. Many tannin components were suggested to be antimicrobial and anticarcinogenic (Chung *et al.,* 1998).

1.4. Peptides

Proteins are high molecular weight polymers of amino acids. A protein may consist of simple chain of polypeptide or several identical chains. Plant proteins are separated in classes based on their solubility or lack of solubility in water, aqueous acid and alkali and 70% alcohol.

1.5. Oils and fatty acids

Essential oils of plants have a large variety of chemical composition having different kinds of compounds which can be extracted by the process of distillation. They contribute to the fragrance of plants and have properties like antibiotic, antitumor, etc. (Bakkali *et al.,* 2008).

2. Review of literature

2.1. Endophytes

Endophytes are an endosymbiotic group of microorganisms. These are bacteria and fungi that dwell in inter- and/or intracellular regions of plants like roots, leaves, stems, etc. (Singh and Dubey, 2015). These microbes can occur within their hosts for their complete lifetime or for some part of their lifecycle without causing any disease to the host and are shown to exhibit complex interactions with their hosts. They may show mutualistic relation, antagonistic relation and sometimes parasitic relations and are known to be ubiquitous in nature (Nair and Padmavathy, 2014). Endophytes are reported to enhance nutrient gain and growth of the host.

Moreover, they have been reported to enhance the ability of the plant to tolerate various types of biotic/ abiotic stresses and insect/ pest and thus assisting in improving its defense mechanism. Endophytes produce bioactive compounds that have significant biotechnological applications including production of pharmaceutical drugs and enzymes (Joseph and Priya, 2011). Endophytic bacteria also help in enhancing the nutrient ability and help in nitrogen fixation for plants. Endophytic bacteria synthesize certain plant hormones that facilitate bryophyte development in plants (Hornschuh *et al.*, 2002).

Table 1. Major milestones in endophytes research

Year	Publications
1898	First symptomless endophyte isolated and cultured from seeds of *Lolium temulentum* (Wilson 1996)
1977-1989	Extensive work on endophytes of conifers (Carroll *et al.,* 1977; Carroll and Carroll, 1978; Carroll and Petrini, 1983)
1982-2000	Significant contribution to knowledge of endophytes (Petrini and Petrini, 1985; Petrini, 1991, 1996)
1996	Endophytic fungi in grasses and woody plants (Redline and Carris, 1996)
1990-2000	Extensive work on endophytes of palms (Rodrigues and Samuels, 1990; Rodrigues, 1994; Taylor *et al.,* 1999; Frohlich *et al.,* 2000)
2000-2008	Significant knowledge on tropical endophytes (Suryanarayanan and Kumaresan, 2000)
2000-2008	Significant contribution to knowledge of endophytes (Arnold *et al.,* 2000; Arnold, 2007)

2.2. Bioactive compounds from endophytes

Shukla *et al.,* 2014 suggested that extraction of metabolites from endophytic microorganisms is affected by a number of factors like climatic condition, the season of sample collection and geographical location of the plant but now it has been proposed that extraction from plants have become easier and efficient (Hussain *et al.,* 2012). It is believed that the production of bioactive substances by endophytes has been directly related to the evolution of the endophytic

microorganisms as endophytes may have included genetic information from plants which eventually helped them in better adaptation within the host plant and protecting the plant from various insects, pathogens and grazing animals (Strobel, 2003).

Medicinal plants are specially known to allow growth of endophytic microorganisms that add to the value of the plant by producing compounds of pharmaceutical importance. Hence it is significant to investigate microflora of medicinal plants. Endophytes interact with other microbial groups that colonize plant tissues like mycorrhiza, pathogens, saprotrophs and epiphytes and are important components of plant microbiomes. (Jain and Pundir, 2015). Natural products like plants extract have so much chemical diversity that they offer limitless opportunities for drug discoveries (Cos *et al.*, 2006). Plants can be used in treatment of chronic as well as infectious diseases as they have a broad variety of bioactive substances of therapeutic potential (Duraipandiyan *et al.*, 2006). The diversity of 2,174 actinobacteria were isolated from medicinal plants collected from tropical rain forests in Xishuangbanna using selective isolation media and methods (Qin *et al.,* 2009). The diversity and beneficial characteristics of endophytic microorganisms have been studied in *Plectranthus tenuiflorus* medicinal plant among 28 bacterial endophytic isolates most isolates exhibited extracellular activity and considerable antimicrobial activity against a range of human pathogenic microorganisms (El-Deeb *et al.,* 2013).

Table 2. Common bioactive compounds isolated from different plant sources and their properties

Bioactive compound	**Plant Source of endophyte**	**Endophyte**	**Property**	**Reference**
Lignans (cathartics, emetics and cholagogue)	*Podophyllum hexandrum*	-	Anticancerous activity	Konuklugil, 1995
Resins (etoposide and teniposide)	*Podophyllum emodi*	-	Anticancerous activity	Konuklugil, 1995

Taxols	*Taxus brevifolia*	*Taxomyces andreanae*	Anticancerous activity	Wani *et al.,* 1971
Ergoflavin	*Mimosops elengi*	PM0651480	Anticancerous activity	Deshmukh *et al.,* 2009
Camptothecin	*Camptotheca acuminate*	*Fusarium solani*	Cytotoxic and antifungal	Joseph and Priya, 2011
Huperzine A	*Huperzia serrata*	*Shiraia sp.*	Cholinesterase inhibitor	Nair and Padmavathy, 2014
Pestalotheol C	-	*Pestalotiopsis theae*	Anti HIV activity	Tian *et al.*, 2008
7-amino-4-methylcoumarin	*Ginkgo biloba*	*Xylaria sp.* YX-28	Food preservative	Liu *et al.,* 2009
Coronamycin	*Monstera sp.*	*Streptomyces sp.*	Anti fungal	Ezra *et al.,* 2004
Chaetomugilin A and D	*Ginkgo biloba*	*Chaetomium globosum*	Antifungal activity	Qin *et al.,* 2009
Pestacin	*Pestalotiopsis microspora*	*Terminalia morobensis*	Antioxidant activity	Harper *et al.,* 2003
Graphislactone A	*Trachelospermum jasminoides*	*Cephalosporium* sp.	Antioxidant activity	Song *et al.,* 2005

2.3. Applications of endophytes and their bioactive compounds

The potential application of endophytes in biotechnological production of flavors has presented recent advances consolidating endophytes as promising biocatalysts (Abrahao *et al.,* 2013). A combination of culture and molecular techniques were used for isolation and identification of endophytes in *Triticum aestivum* to have a comparative study of bacterial endophytes in winter (Robinson *et al.,* 2013). It has been strongly suggested by Heinig *et al.*, 2013 that interactions between the host plant and its inhabiting endophyte both contribute to the coproduction of bioactive molecules.

2.3.1. Anticancer activity

Discovery of Taxol, an anticancerous drug isolated from *Taxus brevifolia,* has been the path breaking success of natural compounds obtained from endophytes (Wani *et al.,* 1971). Anticancerous activity of lignans like emetics, cathartics and cholagogue, isolated from endophyte *Podophyllum hexandrum* have been reported (Konuklugil, 1995). Anticancerous activity of teniposide and etoposide resins obtained from *Podophyllum emodi* has been reported (Konuklugil, 1995). Fungi coded as PM0651480 isolated from the leaves of the plant *Mimosops elengi* has shown good anticancerous activity (Deshmukh *et al.,* 2009).

2.3.2. Antimicrobial activity

Compounds like hydroxypestalopyrone, pestaloside, an aromatic β glucoside, and two pyrones: pestalopyrone having antifungal property were obtained from *Pseudomonas microspora* isolated from *Torreya taxifolia* (Lee *et al*, 1995). Antifungal compound camptothecin was isolated from fungus *Fusarium solani* (Joseph and Priya, 2011). Antifungal property was reported by Oocydin A, Fusaricidin A-D and Coronamycin isolated from endophytes *Serratia marcescens, Paenibacillus polymyxa* and *Streptomyces sp*. respectively (Strobel *et al.*, 2004; Beck *et al.,* 2003; Li *et al.,* 2007; Erza *et al.,* 2007). Antifungal metabolites were obtained from endophytic bacteria isolated from sea grass *Halodule uninervis* (Bibi *et al.*, 2018). Antibacterial activity of endophytic actinomycetes has been reported against *Bacillus cereus* and *Staphylococcus aureus* (Pratiwi *et al.*, 2018). Bactericidal activities of ampicillin, oxacillin, gallic acid, cefalexin and catechin are reported by Akiyama *et al.,* 2001. Antibacterial, antineoplastic, and antiviral properties were also reported in terpenoids (Jalgaonwala *et al.,* 2011; Godstimeetal., 2014). Antibacterial activity of endophytic *Cytonaema sp*. has been reported (Kumar *et al.,* 2014). Strong antibacterial activity against *Leishmania sp.* by polyketide citrinin produced by endophytic fungus *Penicillium janthinellum* inhabiting *Melia azedarach* is reported (Marinho *et al.,* 2005). *Pseudomonas viridiflava*, isolated from grass was found to produce antimicrobial compounds named Ecomycins B and C having the ablity to inhibit human pathogens *Cryptococcus neoformans* and *Candida albicans* respectively (Miller *et al.,* 1998). p-aminoacetophenonic acids isolated from *Streptomyces griseus* inhabiting in *Kandelia candel* also show antimicrobial activities (Guan *et al*., 2005). Antibacterial and antifungal compounds were obtained from endophytes isolated from *Tectona grandis* and *Samanea saman* (Chareprasert *et al.,* 2006). Antibiotics like Munumbicins and

Kakadumycins were isolated from *Streptomyces sp.* (Castillo *et al.,* 2002; 2003). 2,4-diacetylphloroglucinol is an antibiotic against plant pathogens was isolated from endophyte *Pseudomonas fluorescens* Q2-87 (Bangera and Thomashow, 1999).

2.3.3. Antiviral activity-

Cytonic acids A and D are viral inhibitors which were isolated from *Cytomaema sp.* and are reported to inhibit the human cytomegalovirus (Guo *et al.,* 2000). Antiviral drug brefeldin A was isolated from endophytic strain I(R)9-2, *Cladosporium sp*. inhabiting *Quercus variabilis* (Wang *et al.*, 2007).

2.3.4. Antimalarial activity

Munumbicin D and Coronamycin are antimalarial agents isolated from *Streptomyces sp*. inhabiting in plants *Kennedia nigriscans* and *Monstera sp.* respectively (Castillo *et al.,* 2002; Erza *et al.,* 2004).

2.3.5. Antioxidant activity

Pestacin (Harper *et al.,* 2003) and Graphislactone A (Song *et al.*, 2005) isolated from *Terminalia morobensis and Trachelospermum jasminoides* are reported to have antioxidant activities. *Xylaria* sp. inhabiting *Ginkgo biloba* has shown antioxidant properties (Liu *et al.*, 2007).

2.3.6. Drug discovery

Polyphenols produced by *Sauropus androgynus* is reported to reduce fever and *Guazuma ulmifolia* is used in the treatment of liver disease (Steffan *et al.*, 2005). Cholinesterase inhibitor huperzine A (HupA) was isolated from *Huperzia serrata*, (Nair and Padmavathy, 2014).

2.3.7. Biocontrol agents

Isolation, characterization and evaluation of the bioactive products of 74 endophytic bacteria is reported in black pepper (*Piper nigrum*) against *Phytophthora capsici.* Endophytes like *Psuedomonas aeruginosa*, *Pseudomonas putida* and *Bacillus megaterium* have antagonistic property against *Phytophthora* that causes foot rot in black pepper (Aravind *et al.,* 2009). An endophytic fungi *Beauveria bassiana* was found to control the borer insects in sorghum (Tefera and Vidal, 2009) and coffee seedlings (Posada and Vega, 2006). Strong antagonistic property of endophytic bacteria *Bacillus subtilis*, isolated from *Speranskia tuberculata* against *Botrytis cinerea* was reported (Wang *et al.*, 2009). Strains of *Burkholderia pyrrocinia* JK-SH007 and *Burkholderia cepacia* are potential biocontrol agents against poplar canker (Ren *et al.*,

2011). Compounds obtained from *Talaromyces pinophilus* isolated from *Arbutus unedo* displayed toxic effects against the pea aphid *Acyrthosiphon pisum* (Vinale *et al*., 2017).

Table 2. Applications of some endophytic bioactive compounds against pathogenic microorganisms

Bioactive compound	**Compound type**	**Endophyte**	**Inhibits pathogen**	**References**
Cryptocandin A	Cyclic lipopeptide	*Cryptosporiopsis quercina*	*Candida albicans, Trichophyton sp.*	Strobel *et al.,* 1997
Beauvericin	Protein	*Fusarium proliferatum*	*Clostridium botulinum*	Meca *et al.,* 2010
Javanicin	Napthoquinones	*Chloridium sp.*	*Pseudomonas sp.*	Jalgaonwala *et al.,* 2011
Tyrosol and Emodin	Phenol and Quinone	*Diaporthe helianthi*	*Enterococcus hirae* *Salmonella sp.*	Specian *et al.,* 2012
Hypercin	Benzopyrene	*Hypericum perforatum, Streptomyces sp.*	*Salmonella sp.* *Shigella sp.*	Joseph and Priya, 2011; Golinska *et al.,* 2015
Saadamycin		*Cinnamomum mollissimum, Nigrospora sp.*	*Aspergillus niger* *Fusarium oxysporum*	Kaul *et al.*, 2012; El-Gendy and El-Bondkly, 2010; Dutta *et al*., 2014
Valinomycin	Peptide	*Streptomyces tsusimaensis*	*Corona virus*	Alvin *et al.,* 2014

2.3 Selected plant species

Scientific classification (Kokila *et al.,* 2013)

Kingdom	Plantae
(unranked)	Angiosperms
(unranked)	Eudicots
(unranked)	Rosids
Order	Fabales
Family	Fabaceae
Subfamily	Mimosoideae
Tribe	Ingae
Genus	*Albizia*
Specie	lebbeck
Common name	Shirish

Albizia lebbeck, commonly known as Shirish belongs to family Mimosaceae. It is a large, erect, unarmed, deciduous tree is found throughout India. It is generally planted in tropical and subtropical regions of Asia and Africa (Kumar *et al*., 2007). It is a native plant of Rajasthan, fast growing medium sized deciduous tree with a spreading umbrella shaped crown of thin foliage and smoothes, grayish brown bark. The tree best grows on moist, well drained soil. Different parts of *Albizia lebbeck* has shown antidiabetic, antidepressant, analgesic and anti- inflammatory activity, antiulcer properties, antiasthamatic activity, anxiolytic activity, antidiarrhoeal activity, anti-anaphylactic and anti oxidant activity. Bark of the plant is used in the treatment of piles, leucoderma, skin diseases, bronchitis, allergic disorders and asthma. Leaves of the plant strengthen the gums and the teeth and are used in night blindness. The seeds are useful as aphrodisiac and used against gonorrhoea and tuberculosis. Flowers are given for snake-bite and asthma (Sharma and Dubey, 2015).

The plant produces wide variety of bioactive compounds that have numerous pharmacological properties. The bark of the plant is reported to produce compounds that show major antidepressant activity in tests conducted over mice (Velraj *et al.,* 2009). Nootropic and anxiolytic activity of saponins of leaves of the plant on animal models is also reported by Une *et*

al., 2001. Study conducted on animal models showed that anticonvulsant activity is exhibited by ethanolic extracts of leaves of *Albizzia lebbeck* (Kasture *et al.,* 2000).

2.4. Selected classes of bioactive compounds

2.4.1. Alkaloids

Camptothecin, one of the most potent cytotoxic compound is now being obtained from endophytic fungus *Fusarium solani.* Asperfumoid was isolated from an endophytic fungus, *Aspergilus fumigatus* CY018 from the plant *Cynodon dactylon* (Liu *et al.,* 2004).

2.4.2. Steroids/Sterols

Antifungal agent Penicisteroid A is isolated from *Penicillium chrysogenum* (Gao *et al.,* 2013). *Colletotrichum*, a fungal endophyte produces steroids 3β-hydroxy-5α,8α epidioxy-ergosta-6,22-dien, 3β-hydroxy-ergosta-5-ene, 3-oxo-ergosta-4,6,8(14),22-tetraene, 3β,5α-dihydroxy-6β-acetoxy-ergosta-7,22-diene and 3β,5α-dihydroxy-6β-phenylacetyloxy-ergosta-7,22-diene (Lu *et al.,* 2000).

2.4.3. Saponins

Saponins extracted from *Panax notoginseng* are used to treat insomnia and for improving mental function (Xiang *et al.,* 2011). Study of extracts obtained from *Albizzia lebbeck* show significant involvement of gamma-aminobutyric acid (GABA) in the nootropic and anxiolytic activity of saponins obtained from the extract (Une *et al.,* 2001). 19 fungal endophtes were isolated from *Panax ginseng* showing capacity to synthesize saponins (Wu *et al.,* 2013).

2.4.4. Phenolic compounds

Pezicula strain 553, a fungal endophyte, produces 2-methoxy-4-hydroxy-6-methoxymethylbenzaldehyde having antifungal properties against *Cladosporium cucumerinum* (Schulz *et al.,* 1995). p-hydroxyphenylacetic acid, tyrosol, p-coumaric acids and p-Hydroxybenzoic acid are antifungal phenolic acids obtained from *Epichloe typhina*, which can show pathogenic activity against its host *Phleum pratense* (Koshino *et al.,* 1988). Altenusin was isolated from *Alternaria sp.* residing in *Trixis vauthieri* (Cota *et al.,* 2008). Tricin and related flavone glycosides were isolated from *Poa ampla*, infected with a symbiotic fungus *Neotyphodium typhnium* (Ju *et al.,* 1998).

2.4.5. Peptides

Endophytic fungus *Acremonium sp*. produces anticancerous compound Leucinostatin A that colonizes in the plant *Taxus baccata* (Strobel *et al.,* 1997). Echinocandin A having antifungal activity was obtained from endophytic fungi *Cryptosporiopsis* sp. and *Pezicula* sp. inhabiting *Pinus sylvestris* and *Fagus sylvatica* respectively (Noble *et al.,* 1991). Antifungal compound cryptocandin was isolated from the fungus *Candida quercina*, residing in *Tripterigeum wilfordii* (Strobel *et al.,* 1999).

2.4.6. Oils and fatty acids

Oils and fatty acids have been also found as secondary metabolites of endophytic fungi and may have role in crop resistance/susceptibility toward pests (Nicoletti *et al.,* 2015).

3. Objectives

i) Isolation of fungal endophytes from leaf and bark of *Albizia lebbeck*
ii) Screening of extracts of endophytic fungal isolates for bioactive compounds
iii) Characterization of potent fungal endophytes producing bioactive compounds

4. Methodology

i) **Collection of plant material**

Leaf and bark samples of *Albizia lebbeck* will be collected and identified from the herbarium of University of Rajasthan, Jaipur.

ii) **Surface sterilization of leaf and bark of *Albizia lebbeck***

Surface sterilization of the plant material will be done using a modified method of Raviraja, 2005. The sample(s) will be immersed in varied concentrations of ethanol and sodium hypochlorite solution for different time duration followed by rinsing with sterile distilled water and will be allowed to surface dry under sterile conditions.

iii) **Isolation of fungal endophytes from leaf and bark of *Albizia lebbeck***

Endophytes will be isolated by placing the dry and sterile plant parts on petri plates containing potato dextrose agar medium (PDA) supplemented with streptomycin to suppress bacterial growth followed by incubation at 30°C in a chamber for up to one week (Bhardwaj *et al.,* 2015).

iv) **Characterization of fungal endophytes**

Characterization will be done on the basis of vegetative and sporulating structures by lacto phenol staining (Cappuccino and Sherman, 1999).

v) **Bioactive compound production and extraction**

Endophytic fungal isolate will be grown in broth medium under stationary condition for 21(approximately) days at room temperature. After incubation culture broth will be filtered. Bioactive compound will be extracted using different solvents like alcohol, water, n-hexane and chloroform. The extract will be concentrated and stored at 4°C for further use (Radji *et al.,* 2011).

vi) **Screening of extracts**

Screening of prepared extracts for bioactive compounds like alkaloids, phenolic compounds flavanoids and tannins, terpenoids steroids and sterols, fixed oils and fatty acids and protein and amino acids will be done according to the standard methods given by Al *et al.,* 2010; Kokate *et al.,* 2009; Harborne, 1998 (Table 3).

Table 3. Screening tests for bioactive compounds

Name of compound	Reagent used	Indication of positive result
Alkaloids	HCl, Mayer's reagent	Creamish precipitate
Terpenoids i) Steroids ii) Saponins	$CHCl_3$, H_2SO_4 Libermann-Burchard reaction -	Reddish-brown precipitate Blue-green ring Emulsion formation
Phenolic compounds i) Flavonoids ii) Tannins	$FeCl_3$ NaOH $FeCl_3$, H_2SO_4	Dark green colour Yellow to colourless Yellowish-brown precipitate.
Peptides	Million's reagent	White precipitate
Oils and fatty acids	KOH, Phenolphthalein	Soap formation

vii) **Quantification and fractionation of bioactive compounds**

Quantification and fractionation of bioactive compounds from potent fungal endophyte extract will be carried out by HPLC.

viii) **Characterization of potent fungal endophyte**

The potent fungal isolate will be characterized by ITS sequencing.

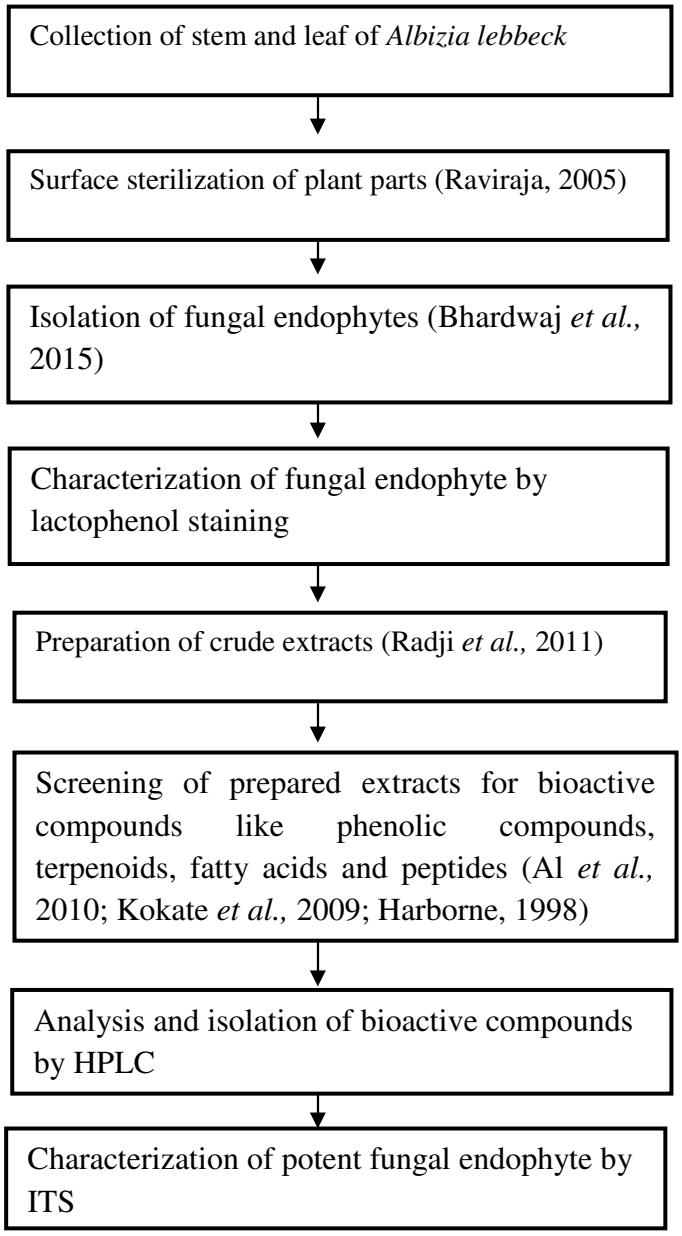
Collection of stem and leaf of *Albizia lebbeck*
Surface sterilization of plant parts (Raviraja, 2005)
Isolation of fungal endophytes (Bhardwaj *et al.,* 2015)
Characterization of fungal endophyte by lactophenol staining
Preparation of crude extracts (Radji *et al.,* 2011)
Screening of prepared extracts for bioactive compounds like phenolic compounds, terpenoids, fatty acids and peptides (Al *et al.,* 2010; Kokate *et al.,* 2009; Harborne, 1998)
Analysis and isolation of bioactive compounds by HPLC
Characterization of potent fungal endophyte by ITS

Fig. 1. Outline of work for proposed study

5. Significance and probable outcome

i) The need for novel and useful compounds to offer relief and assistance in various aspects of human condition is ever growing. Endophytes are comparatively less studied and possible sources of variety of natural products for utilization in medicine, agriculture, and industry. Consequently, the opportunity to find new and interesting endophytic microorganisms among myriads of plants in different settings and ecosystems is great. Moreover, bioprospecting of the endophytes for productionof bioactive compounds may greatly add to the research and development for human welfare.

ii) The selected plant species *Albizia lebbeck* is a native of Rajasthan with medicinal value and very less work is reported in literature in regards with endophytic bacteria of the plant. Thus, it would be significant to study the production of bioactive compounds by the endophytes.

6. Conclusion

Endophytes are a very less investigated group of microorganisms having the ability to synthesize bioactive compounds that have variety of applications. They are the sources of discovery of new compounds for novel drug discovery and can act as antimicrobial, antifungal, antiviral, antioxidant, anti inflammatory agents, etc. Hence, it is of vital importance to draw the attention of researchers toward this promising field and it's possible exploration in various fields like medical, pharmaceutical, food and cosmetics.

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